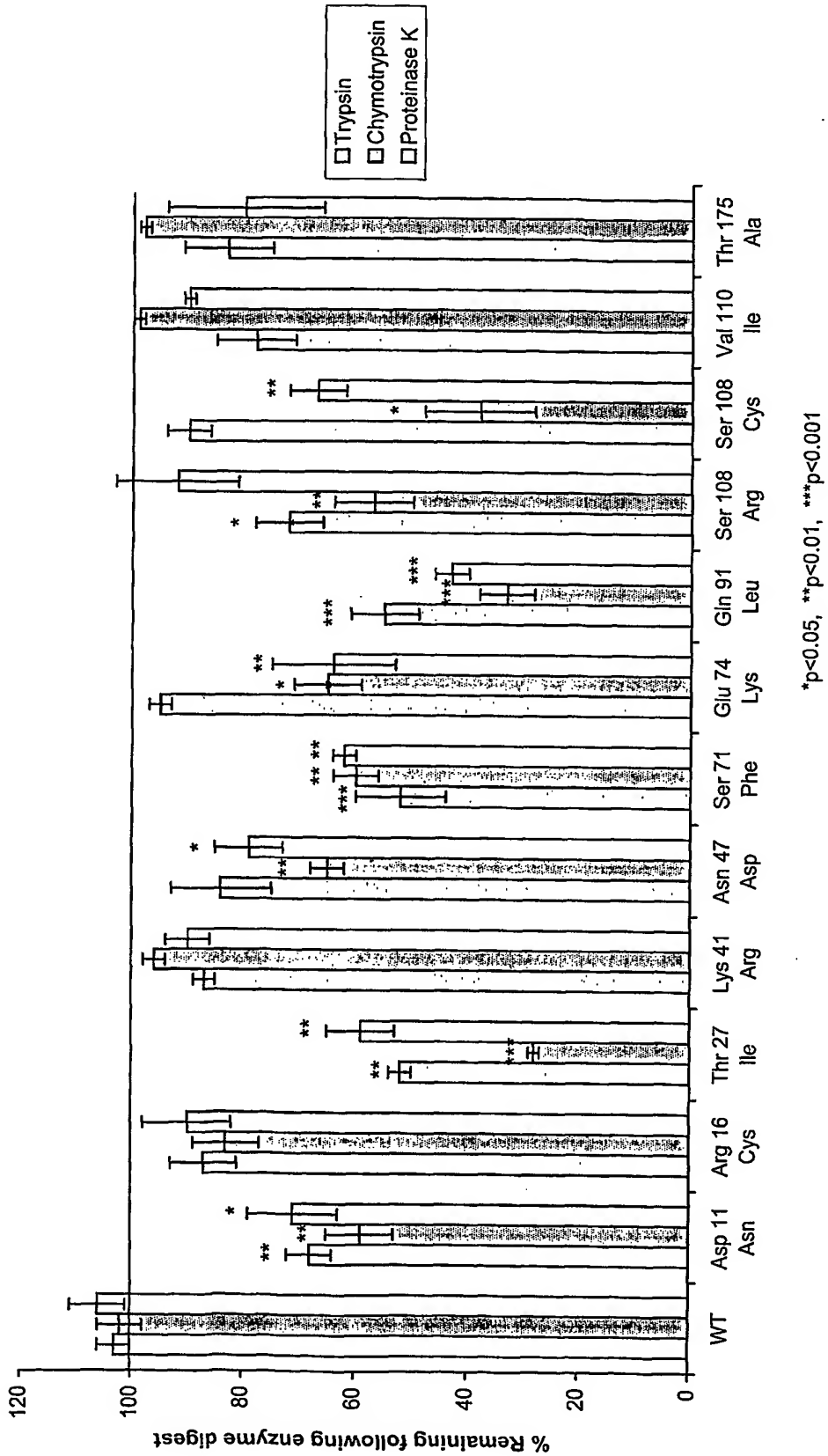
Enzyme Analysis of GH Variants
% Remaining following enzyme digest
120
100
80
60
40
20
0
Trypsin
Chymotrypsin
Proteinase K
WT
Asp 11 Asn
Arg 16 Cys
Thr 27 Ile
Lys 41 Arg
Asn 47 Asp
Ser 71 Phe
Glu 74 Lys
Gln 91 Leu
Ser 108 Arg
Ser 108 Cys
Val 110 Ile
Thr 175 Ala
*p<0.05, **p<0.01, ***p<0.001

FIGURE 1

LOCATION OF AMINO ACID RESIDUES INVOLVED IN MISSENSE MUTATIONS AND THE PREDICTED CONSEQUENCES OF SUBSTITUTION

Amino Acid Substitution	Location of amino acid residues, interactions and consequences of substitution
D11N	D11 (helix 1) is solvent accessible. No obvious adverse consequences of substitution by N.
R16C	R16 (helix 1) is solvent accessible and interacts with site 2 andE44 & W169 of GHR. Substitution by C could adversely affect site 2 binding. Introduction of unpaired cysteine could also lead to inter-molecular disulphide bridging with unpaired cysteine could also lead to inter-molecular disulphide bridging with consequent protein aggregation.
T27I	T27 lies in helix 1 but is buried. Substitution by I may affect internal packing around helix 1 and the loop between helix 2 and helix 3.
K41R	K41 (loop 1 lies between helices 1 and 2) is solvent accessible. K41 N atom exhibits ionic interaction with GHR E127 Oε2 and has been implicated in GHR binding by alanine scanning mutagenesis. Substitution by R may conserve ionic interaction but could cause unfavourable steric interactions.
N47D	N47 (loop 1) is solvent accessible. No interaction with GHR. No obvious adverse consequences of substitution by D.
S71F	S71 (loop 1) is solvent accessible. Hydrophobic side-chain of substituting F could decrease protein solubility and affect folding.
E74K	E74, partially exposed with N-terminal of helix 2, may interact with Q137 thereby stabilising helix 2. Introduction of K may affect helix stability.
Q91L	Q91 located at C-terminal end of helix 2. Introduction of L increases hydrophobicity and may affect solubility and folding.
S108R	S108 (helix 3) is solvent accessible but does not interact with GHR. Substitution by R could adversely affect helix formation.
S108C	S108 (helix 3) is solvent accessible. Introduction of unpaired cysteine could lead to inter-molecular disulphide bonding with consequent protein aggregation.
V110I	V110 (N-terminal of helix 3) is deeply buried in hydrophobic core. Conservative substitution but I has longer sidechain and may encounter steric hindrance.
T175A	T157 (helix 4) is solvent accessible and has been implicated in GHR binding by alanine scanning mutagenesis. T175 forms H-bond with D171 of GH and W169 and R43 of GHR. Introduction of A may destabilise helix thereby decreasing receptor binding.

FIGURE 2